REMARKS

Claims 1-3 and 5-22 are pending in this application. Claim 4 has been cancelled without prejudice or disclaimer to the subject matter contained therein.

SPECIES ELECTION

The Examiner has required a species election in conjunction with the election of Group I drawn to claims 1-3 and 5-21 in the Restriction Requirement dated December 12, 2007. Applicants respectfully submit a full and complete response.

Subgroup 1: Species of Compound of Molecules

Applicants respectfully traverse this species election requirement.

The Examiner has required a species election within claim 1 to a method for detecting only one of the compounds which are recited. The Examiner states that proteins and carbohydrates do not share a common structure (see, paragraph 14 of the Restriction Requirement dated December 12, 2007), and therefore he regards the detection of proteins and carbohydrates, as well as the other components recited in claim 1, to not be linked so to form a single general inventive concept under PCT Rule 13.1.

Furthermore, the Examiner refers to MPEP 1850 in support of the requirement, which states that unity of invention may be denied in cases where different compounds do not share a common structure (see MPEP 1850 III B (1)). However, in the present case at least the proteins, the peptides and the nucleic acids named in claim 1 do share a common property within the meaning of MPEP 1850 III A, despite having different chemical structures, in that all of these components can be detected by generic silver staining methods commonly known and described in the prior art.

Applicants respectfully submit that the attached articles ("Silver Stain" – ATTACHMENT A; "Gel Electrophoresis" – ATTACHMENT B) show that both proteins and nucleic acids are detectable by staining methods. The difference between a protein and a peptide is only that the protein has a larger molecular mass. Chemically there is no difference, both consist of amino acids. For one of skill in the art it is clear that by silver staining methods every kind of biomolecules can be detected.

On these bases, Applicants respectfully request the Examiner to reconsider and withdraw the species election requirement of subgroup I and provisionally elect proteins. Applicants respectfully submit that claims 1-3 and 5-21 are encompassed by the elected species.

Subgroup 2: Species of Compound of Bifunctional Agent

Applicants elect as bifunctional agent a compound with the general formula R-X, wherein X is the reducing moiety and R is the hydrophobic moiety, wherein the hydrophobic moiety is an acyloxy-radical and the reducing moiety is a vitamin having antioxidative properties. Applicants respectfully submit that the elected claims are encompassed by all pending claims. Applicants respectfully submit that claims 1-3 and 5-21 are encompassed by the elected species.

Subgroup 3: Species of Metal Compound

Applicants respectfully traverse this rejection.

One of even rudimentary skill in the art knows that every silver compound is suitable as long as it is able to provide silver ions which can be reduced during the developing step. Applicants respectfully submit that the elected claims are encompassed by all pending claims. Applicants respectfully submit that claims 1-3 and 5-21 are encompassed by the elected species.

However, Applicants provisionally elect silver nitrate.

Subgroup 4: Species of Solid Support if Used

Applicants respectfully elect polyacrylamide. Applicants respectfully submit that the elected claims are encompassed by all pending claims.

Subgroup 5: Species of Compound Alcoholic Solution

Applicants respectfully elect ethanol. Applicants respectfully submit that the elected claims are encompassed by all pending claims.

Subgroup 6: Species of Compound Complexing Agent

Applicants respectfully elect EDTA. Applicants respectfully submit that the elected claims are encompassed by all pending claims.

Subgroup 7: Species of Compound of Developing Solution

Applicants respectfully elect sodium thiosulfate. Applicants respectfully submit that the elected claims are encompassed by all pending claims.

CONCLUSION

In view of the foregoing, Applicants respectfully submit that a full and complete response has been made.

The Examiner is welcomed to contact the undersigned attorney at the below-listed number and address with any questions or comments regarding this matter.

Respectfully submitted,

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Date: January 16, 2009

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Silver stain

ATTACHMENT A

From Wikipedia, the free encyclopedia

Silver staining is the use of silver to stain histologic sections. This kind of staining is important especially to show proteins (for example type III collagen) and DNA. It is used to show both substances inside and outside cells. Silver staining is also used in temperature gradient gel electrophoresis and in polyacrylamide gels.

Some cells are *argentaffin*. These reduce silver solution to metallic silver after formalin fixation. Other cells are *argyrophilic*. These reduce silver solution to metallic silver after being exposed to the stain that contains a reductant, for example hydroquinone or formalin.

Silver nitrate forms insoluble silver phosphate with phosphate ions. When subjected to a reducing agent, usually hydroquinone, it forms black elementary silver. This is used for study of formation of calcium phosphate particles during bone growth.

Silver staining is used in light microscopy. The metallic silver particles are deposited on sensitised reticulin fibres and are then easily seen in the microscopic preparations.

Silver stain aids in the perception of reticular fibers.

Use in art

Silver staining is also a technique in traditional stained glass to produce the yellow, brown or amber shading when painting on glass. It is a technique that is often used for realistic hair colors. It was discovered in the 14th Century but was not originally used very frequently.

Organisms highlighted

Pseudomonas, Legionella

External links

- MedEd at Loyola *Histo/practical/stains/hp2-55.html (http://www.meddean.luc.edu/Lumen/MedEd/Histo/practical/stains/hp2-55.html)*

Retrieved from "http://en.wikipedia.org/wiki/Silver_stain"
Categories: Staining | Electron microscopy stains

ATTACHMENT B

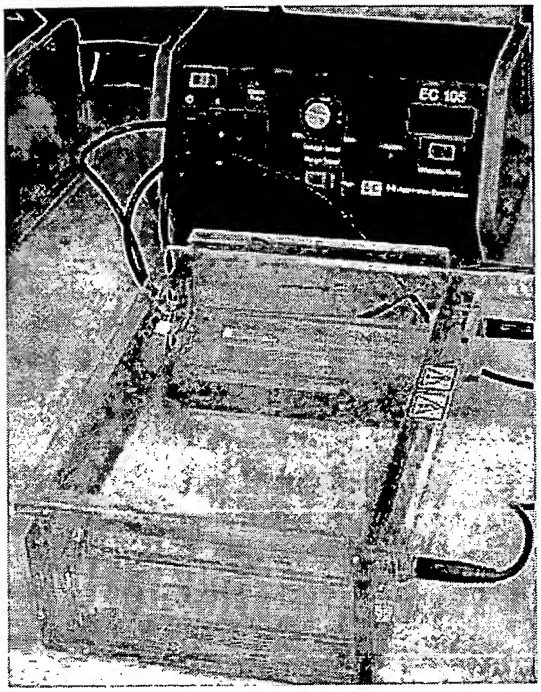
Gel electrophoresis

From Wikipedia, the free encyclopedia

Gel electrophoresis is a technique used for the separation of deoxyribonucleic acid, ribonucleic acid, or protein molecules using an electric current applied to a gel matrix.[1] It is usually performed for analytical purposes, but may be used as a preparative technique prior to use of other methods such as mass spectrometry, RFLP, PCR, cloning, DNA sequencing, or Southern blotting for further characterization.

Contents

Gel electrophoresis



Gel electrophoresis apparatus - An agarose gel is placed in this buffer-filled box and electrical current is applied via the power supply to the rear. The negative terminal is at the far end (black wire), so DNA migrates toward the camera.

Classification Electrophoresis

Other Techniques

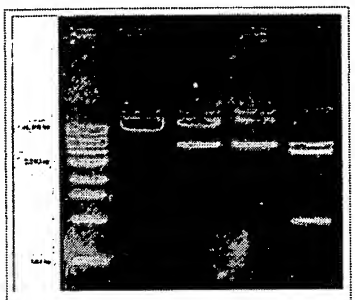
Related Capillary electrophoresis
SDS-PAGE
Two-dimensional gel electrophoresis
Temperature gradient gel electrophoresis

Separation

The term "gel" in this instance refers to the matrix used to contain, then separate the target molecules. In most cases the gel is a crosslinked polymer whose composition and porosity is chosen based on the specific weight and composition of the target to be analyzed. When separating proteins or small nucleic acids (DNA, RNA, or oligonucleotides) the gel is usually composed of different concentrations of acrylamide and a cross-linker, producing different sized mesh networks of polyacrylamide. When separating larger nucleic acids (greater than a few hundred bases), the preferred matrix is purified agarose. In both cases, the gel forms a solid, yet porous matrix. Acrylamide, in contrast to polyacrylamide, is a neurotoxin and must be handled using appropriate safety precautions to avoid poisoning.

"Electrophoresis" refers to the electromotive force (EMF) that is used to move the molecules through the gel matrix. By placing the molecules in wells in the gel and applying an electric current, the molecules will move through the matrix at different rates, usually determined by their mass to charge ratio (Z), toward the anode if negatively charged or toward the cathode if positively charged [2].

Visualization



I Agarose gel prepared for DNA analysis - The first lane contains a *DNA ladder* for sizing, and the other four lanes show variously-sized DNA fragments that are present in some but not all of the samples.

After the electrophoresis is complete, the molecules in the gel can be stained to make them visible. Ethidium bromide, silver, or coomassie blue dye may be used for this process. Other methods may also be used to visualize the separation of the mixture's components on the gel. If the analyte molecules fluoresce under ultraviolet light, a photograph can be taken of the gel under ultraviolet lighting conditions. If the molecules to be separated contain radioactivity added for visibility, an autoradiogram can be recorded of the gel.

If several mixtures have initially been injected next to each other, they will run parallel in individual lanes. Depending on the number of different molecules, each lane shows separation of the components from the original mixture as one or more distinct bands, one band per component. Incomplete separation of the components can lead to overlapping bands, or to indistinguishable smears representing multiple unresolved components.

Bands in different lanes that end up at the same distance from the top contain molecules that passed through the gel with the same speed, which usually means they are approximately the same size. There are molecular weight size markers available that contain a mixture of molecules of known sizes. If such a marker was run on one lane in the gel parallel to the unknown samples, the bands observed can be compared to those of the unknown in order to determine their size. The distance a band travels is approximately inversely proportional to the logarithm of the size of the molecule.

Applications

Gel electrophoresis is used in forensics, molecular biology, genetics, microbiology and biochemistry. The results can be analyzed quantitatively by visualizing the gel with UV light and a gel imaging device. The image is recorded with a computer operated camera, and the intensity of the band or spot of interest is measured and compared against standard or markers loaded on the same gel. The measurement and analysis are mostly done with specialized software.

Depending on the type of analysis being performed, other techniques are often implemented in conjunction with the results of gel electrophoresis, providing a wide range of field-specific applications.

Nucleic acids

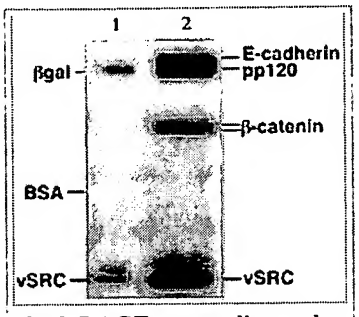
In the case of nucleic acids, the direction of migration, from negative to positive electrodes, is due to the naturally-occurring negative charge carried by their sugar-phosphate backbone.[3]

Double-stranded DNA fragments naturally behave as long rods, so their migration through the gel is relative to their radius of gyration, or, for non-cyclic fragments, size. Single-stranded DNA or RNA tend to fold up into molecules with complex shapes and migrate through the gel in a complicated manner based on their tertiary structure. Therefore, agents that disrupt the hydrogen bonds, such as sodium hydroxide or formamide, are used to denature the nucleic acids and cause them to behave as long rods again.[4]

Gel electrophoresis of large DNA or RNA is usually done by agarose gel electrophoresis. See the "Chain termination method" page for an example of a polyacrylamide DNA sequencing gel.

Proteins

Proteins, unlike nucleic acids, can have varying charges and complex shapes, therefore they may not migrate into the gel at similar rates, or at all, when placing a negative to positive EMF on the sample. Proteins therefore, are usually denatured in the presence of a detergent such as sodium dodecyl sulfate/sodium dodecyl phosphate (SDS/SDP) that coats the proteins with a negative charge.[1] Generally, the amount of SDS bound is relative to the size of the protein (usually 1.4g SDS per gram of protein), so that the resulting denatured proteins have an overall negative charge, and all the proteins have a similar charge to mass ratio. Since denatured proteins act like long rods instead of having a complex tertiary shape, the rate at which the resulting SDS coated proteins migrate in the gel is relative only to its size and not its charge or shape.[1]



SDS-PAGE autoradiography - The indicated proteins are present in different concentrations in the two samples.

Proteins are usually analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), by native gel electrophoresis, by quantitative preparative native continuous polyacrylamide gel electrophoresis (QPNC-PAGE), or by 2-D electrophoresis.

History

- 1930s - first reports of the use of sucrose for gel electrophoresis
- 1955 - introduction of starch gels, mediocre separation
- 1959 - introduction of acrylamide gels (Raymond and Weintraub); accurate control of parameters such as pore size and stability
- 1964 - disc gel electrophoresis (Ornstein and Davis)
- 1969 - introduction of denaturing agents especially SDS separation of protein subunit (Weber and Osborn)[5]
- 1970 - Laemmli separated 28 components of T4 phage using a stacking gel and SDS
- 1975 - 2-dimensional gels (O'Farrell); isoelectric focusing then SDS gel electrophoresis
- 1977 - sequencing gels
- late 1970s - agarose gels
- 1983 - pulsed field gel electrophoresis enables separation of large DNA molecules
- 1983 - introduction of capillary electrophoresis

A 1959 book on electrophoresis by Milan Bier cites references from the 1800s.[6] However, Oliver Smithies made significant contributions. Bier states: "The method of Smithies ... is finding wide application because of its unique separatory power." Taken in context, Bier clearly implies that Smithies' method is an improvement.

See also

- Two-dimensional gel electrophoresis
- DNA electrophoresis
- Electrofocusing
- Gel isolation
- Native Gel Electrophoresis
- Northern blotting
- Protein electrophoresis
- SDS PAGE
- Southern blotting
- Western blotting
- Zymography

References

1. ^ [a] [b] [c] Berg JM, Tymoczko JL Stryer L (2002). *Molecular Cell Biology* (5th ed. ed.). WH Freeman. ISBN 0-7167-4955-6. http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=stryer.section.438#455.
2. ^ Robyt, John F.; White, Bernard J. (1990). *Biochemical Techniques Theory and Practice*. Illinois: Waveland Press. ISBN 0-88133-556-8.
3. ^ Lodish H, Berk A, Matsudaira P, *et al* (2004). *Molecular Cell Biology* (5th ed. ed.). WH Freeman: New York, NY. ISBN 978-0716743668. http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=mcb.section.1637#1648.
4. ^ Troubleshooting DNA agarose gel electrophoresis. Focus 19:3 p.66 (1997).
5. ^ http://www.ncbi.nlm.nih.gov/pubmed/5806584
6. ^ Milan Bier (ed.) (1959). *Electrophoresis. Theory, Methods and Applications* (3rd printing ed.). Academic Press. pp. 225. LCC 59-7676. OCLC 1175404.

External links

- Biotechniques Laboratory electrophoresis demonstration (http://gslc.genetics.utah.edu/units/biotech/gel) , from the University of Utah's Genetic Science Learning Center
- Discontinuous native protein gel electrophoresis (http://www3.interscience.wiley.com/cgi-bin/abstract/113343976/ABSTRACT)

Retrieved from "http://en.wikipedia.org/wiki/Gel_electrophoresis"
Categories: Protein methods | Molecular biology | Laboratory techniques | Electrophoresis | Polymerase chain reaction